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Up-concentration of succinic acid, lactic acid, and ethanol fermentations broths by forward osmosis

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Highlights

- Succinic acid, lactic acid, and ethanol were produced from residual resources
- Forward osmosis was examined to up-concentrate fermentation products
- Succinic acid concentration factor of 5.0 was achieved
- Best results were obtained with NaCl 5.0 M as draw solution
- Water removals of up to 85% were attained

Abstract

The potential of Forward Osmosis (FO) technology to up-concentrate succinic acid (HSuc), lactic acid (HLac), and ethanol (Eth) from fermentation broths was investigated. The aforementioned molecules were obtained from two residual resources (biopulp and algal biomass) *via* anaerobic fermentation. HLac and Eth were produced from biopulp and HSuc was produced from macroalgal biomass hydrolysate. Herein, HLac, Eth, and HSuc titers of 14.98 ± 0.76 , 19.11 ± 0.51 and 38.8 ± 0.32 g L⁻¹ were obtained respectively. After treatment with centrifugation, FO was applied to the treated broths using a thin film composite hollow fibre (TFHF) membrane. Best results were obtained with the HSuc fermentation broth using NaCl 5.0 M as draw solution. Final HSuc titer of 186.7 ± 9.3 g L⁻¹ and water removal of 85% were attained. Findings in this work highlight a novel application of TFHF membranes.

Key words

Forward osmosis; Hollow fibre membrane; Downstream separation; Lactic acid; Succinic acid, Biopulp; *Saccharina latissima*

1 Introduction

In a petroleum based economic model, the need of oil supply and the market fluctuations produce continuous instability and geopolitical issues [1]. Currently, oil-based refineries are essential to produce the daily consumer goods and fuels. However, a transition towards a sustainable society is compulsory to meet the Sustainable Development Goals and avoid the climate change. In the mid-term future, biorefineries will constitute an attractive platform to develop a bio-based economy, since these are analogous to oil-based refineries, where a number of integrated processes are used with biomass as feedstock to produce energy, fuels, biomaterials and a variety of chemicals, thus reducing the dependence on fossil fuels [2]. Among different molecules, lactic acid and succinic acid were envisioned as key platform chemicals [3,4]. As shown in **Table 1**, these chemicals are used in a wide range of applications and their market growth prospects are promising. In addition, these can be produced by means of energy efficient fermentation processes[5–9] from different organic wastes rich in carbohydrates [10,11], thereby avoiding the use of edible crops. By far one of the most prominent succinic acid producing wild strain is *Actinobacillus succinogenes* [12]. Particularly, the *A. succinogenes* FZ53 mutant strain has been reported to produce the highest succinate titer using glucose with a yield and productivity of 0.82 g of succinic acid g⁻¹ of glucose and 1.36 g L⁻¹ h⁻¹, respectively [13]. *A. succinogenes* remains an attractive production strain and one of its main advantages is that can metabolise most naturally occurring sugars [14]. For instance, Marinho et al.[9] reported that *A. succinogenes* was able to perform co-fermentation of glucose and mannitol when macroalgal biomass was used as substrate.

As regards to lactic acid production, *Lactobacillus delbrueckii* has been reported to produce mainly the isomer L-lactic acid, which is desirable for PLA production and other commercial

applications [15]. This bacterial strain can produce significant amounts of ethanol during fermentation of carbohydrates, which is a valuable biofuel (Table 1). Fermentation performance is highly affected when inhibition takes place due to main product and/or by-products accumulation. Furthermore, product recovery from the fermentation broth can account for 50-80% of the overall process cost [16,17]. To this respect, it is imperative to develop downstream separation processes and technologies in order to enhance the cost-effectiveness and competitiveness of the process. For instance, precipitation methods with calcium salts have been used for the recovery of succinic acid from the fermentation broth [18]. However, the high consumption of $\text{Ca}(\text{OH})_2/\text{CaCO}_3$ and by-products generation are undesirable from an waste management view point. Li et al. [20] proposed one-step direct crystallization process for succinic acid recovery from the fermentation broth. Despite, the by-products present in the fermentation broth, i.e. lactic acid, acetic acid, and formic acid; recovery and purity of succinic acid of 70% and 90% were attained, respectively. Similarly, high consumption of sulfuric acid, generation of a non-soluble waste gypsum cake, and impurities present in the final product were associated to lactic acid based precipitation process [21]. Distillation constitutes another traditional technology, however with the inconvenience of high energy demand due to the high water content of the fermentation broth [22]. Furthermore, solvent extraction has been applied to upgrade the fermentation mixtures where high distribution coefficients are required. Herein, high molecular weight amines have been identified as promising extractants [23]. However, the main obstacle of solvent extraction is that requires high specific area of separation and an additional stripping unit to recover the solvent resulting in higher investment and operational costs. Process unit operations such as membrane-based process i.e. pervaporation, nanofiltration, electrodialysis, or combination of the latter have been proposed with the aim to abate the energy

consumption of the downstream process [24–27]. Recently, Mai et al. [28] combined microfiltration, nanofiltration and pervaporation-assisted esterification for the production of high purity D-lactic acid from starch. During the pervaporation, ethanol was used to produce ethyl lactate, with a water removal of 95% resulting in a lactic acid conversion yield of 0.93. The process was completed by distillation and hydrolysis with deionized water and a final polymerization into poly(D-lactide). Ma et al. [29] recovered lactic acid from stillage by means of bipolar membrane electrodialysis resulting in product recovery yields of 71.2%. On the other hand, Sosa et al. [27] combined a three step membrane process including, electrodialysis, nanofiltration, and Donnan dialysis, to recover succinic acid from carob pod-based fermentation broths. Although rejections above 90% for succinate were obtained, technical difficulties associated to membrane fouling and reduced fluxes were reported. In line with other studies Thuy and Boontawan [30] proposed a multi-stage process that consisted of microfiltration and nanofiltration-assisted crystallization to recover high purity succinic acid (i.e. 99.18%). Yet despite these promising achievements in the downstream process, the proposed downstream design needs to be environmentally friendly, minimizing energy and chemicals consumption, and to fulfill the potential characteristics to be feasible on a commercial level [31]. Removal of water from fermentation broth could be highly beneficial for the overall energy economy of the process, for example, when an energy intensive separation step, i.e. distillation, is required. As reported, product titers of at least of 120 g L^{-1} are required to diminish the cost of the product recovery. With this in mind, Forward Osmosis (FO) technology possesses significant advantages over other membrane-based technologies, i.e. nanofiltration or reverse osmosis, due to the low membrane fouling and low energy requirement [32]. FO uses the natural water flux that is established due to the osmotic pressure gradient between the feed solution and draw solution,

which contain different saline concentrations [33,34]. The water flux across the membrane in FO, dilutes the draw solution and results in an up-concentration effect in the feed side. The application of FO technology with fermentation mixtures, could lead to up-concentrate the target chemicals which is valuable for subsequent downstream [35]. Interestingly, osmotic distillation has positively been applied to up-concentrate pomegranate juice where the quality parameters of the juice were improved [36]. Newly, the application of FO membranes to recover fermentation products is gaining interest due to the aforementioned advantages. Law et al. [16] obtained promising results regarding the succinic acid recovery with FO assisted crystallization process. The authors used cellulose triacetate flat sheet membranes and reported solute concentration factors of 3.9 with a succinic acid titer of 111.16 g L⁻¹. The crystallization led to a purity and yield of succinate crystals of 90.52% and 67.09% respectively. Nonetheless, the application of FO with real fermentation broths demands a thorough study for further evaluation of the technology. For example by evaluating different types of membranes, or testing additional draw solutes than NaCl [37] and different operation regimes to minimize internal concentration polarization (ICP) and reverse solute flux. In this study, an osmotic pressure driven process with a thin film composite hollow fibre (TTHF) membrane was evaluated to treat real fermentation broths containing organic compounds of industrial importance, namely, succinic acid, lactic acid, and ethanol. Fermentation experiments were performed to obtain the fermentation broths that were used as feed solution in FO tests. A straightforward downstream process was employed, where the broths were centrifuged to remove the cell mass and debris, but not further pretreatments were performed prior to FO. Different concentrations of NaCl, i.e. 1.5 M and 5.0 M, were used as draw solution. During the FO tests, process parameters were evaluated, such as the water flux, the rejection rate of the membranes, the water and the solute concentration factor.

2 Materials and Methods

2.1 Fermentation substrates

Biopulp consisted of source separated municipal organic waste, supermarket waste and food residues from restaurants and canteens. After collection, biopulp was pretreated with a biopulper equipment [38] to release the biodegradable organics and sort out the non-degradable fraction (e.g. plastics, metals, etc.). The resulting pretreated biopulp was picked up at HCS A/S and transported to our laboratory facilities. Furthermore, HCS A/S also provided a residual liquid stream from the biopulping process. The pretreated biopulp was diluted with distilled water to reach a volumetric ratio of 1:1; thereafter both substrates were stored immediately at $-20\text{ }^{\circ}\text{C}$ for subsequent characterization and experiments. The characteristic of the diluted biopulp and the residual liquid stream are shown in **Table 2**.

2.2 Chemicals and inoculum

All chemicals used in this study were of analytical grade and were purchased from Sigma Aldrich ApS (Brøndby, Denmark). The strain of *Lactobacillus delbrueckii* (DSM 20074) was obtained from DSMZ (German Collection of Microorganisms and Cell Cultures). The culture stock was stored in glycerol at $-80\text{ }^{\circ}\text{C}$ prior to use. Initially, the microbes were grown at $37\text{ }^{\circ}\text{C}$ for 24 h in 20 mL flasks at a concentration of 10% (v/v) along with 90% (v/v) of sterilized MRS medium. For every subsequent generation, the *Lactobacilli* culture was transferred to 100 mL serum bottles under the same operational conditions as the first generation. The initial pH in all flasks was adjusted to 6.2 with NaOH 8.0 M solution.

2.3 Fermentation experiments

Succinic acid fermentation broth from *Saccharina latissima* macroalgal hydrolysate was previously obtained by [39] and therefore was used directly for the forward osmosis tests. On the other hand, biopulp has been proven to be a promising feedstock for lactic acid production and biofuels such as ethanol [40,41] and therefore was used for this purpose.

2.3.1 Lactic acid production

Batch fermentations were performed in duplicates in two identical 3.0 L fermenters (Sartorius BIOSTAT Aplus, Germany) with an initial working volume of 2.0 L. Fermenters were filled out with residual liquid stream from biopulp -after separating the solid residue by centrifugation -and pH was adjusted to 6.2 and hereinafter kept at this value with NaOH 8.0 M solution over the course of the fermentation (27 h). Fermenters were sparged with N₂ gas for 10 min to create anaerobic conditions and inoculation was performed –just prior temperature reached 37 °C –with 10% (v/v) of exponentially growing *Lactobacillus delbrueckii* inoculum at mesophilic (37 °C) conditions. Samples were collected every 2 – 10 h and stored at –20 °C prior to HPLC analysis.

2.3.2 Ethanol production

Batch fermentations for ethanol production were performed in the aforementioned fermenters at same conditions as for lactic acid experiments but in this case diluted biopulp (1:1) was used as substrate and fermentation time was 14 h. Inoculation was performed with 10% (v/v) of exponentially growing *Lactobacillus delbrueckii* inoculum at 37 °C. It should be pointed out that this strain can also produce Eth as main fermentation product [47]. Samples were collected every 2 – 10 h and stored at –20 °C prior to HPLC analysis. Fermentation broths from lactic acid and

ethanol fermentations were centrifuged to recover the liquid fraction and then stored them at -20 °C prior to further treatment with forward osmosis.

2.3.3 Succinic acid production

Succinic acid fermentation broth was obtained from Marinho et al. [9]. Authors performed batch fermentation tests with macroalgal hydrolyzate. Prior to fermentation tests, enzymatic hydrolysis of macroalgal biomass *Saccharina latissima* was performed for 48 h, at 50 °C and 150 rpm. After completion of the enzymatic hydrolysis, the macroalgal slurry was centrifuged to recover the liquid fraction, namely, macroalgal hydrolysate which was used for fermentation experiments at 37 °C with the bacterial strain *Actinobacillus succinogenes* 130Z. Thereafter the fermentation broth was centrifuged to recover the liquid fraction, which was stored at -20 °C. Detailed explanation of the experimental set up can be found in Marinho et al. [9]. The liquid fraction from the centrifugation step was used to perform the forward osmosis experiments.

2.4 Forward osmosis (FO) experiments

Forward osmosis tests were performed with the liquid fraction obtained by centrifugation of the fermentation broths aforementioned. The membranes used were purchased from Aquaporin A/S and consisted of thin film composite hollow fiber membranes with a surface area of 0.3 m^2 and a water flux $> 7 \text{ L m}^{-2} \text{ h}^{-1}$, when 1.0 M NaCl solution is used as draw solution and DI water as feed solution with 300 mL min^{-1} flowrate. The hollow fibre membranes have biomimetic protein channels incorporated into proteoliposomes layer supported by a microporous structure [42]. The aquaporin proteins are water selective and can operate with turnover rates up to 109 water molecules per second [43], which translates into a fast permeability.

During FO tests, the 1 L feed solution reservoir contained the liquid fraction of the fermentation broth, while the 4 L draw solution reservoir contained NaCl solution of 1.5 or 5.0 M, [16,44] respectively (**Table 3**). NaCl was employed as draw solute, since is inexpensive, nontoxic, produces a high osmotic pressure and is easy to be regenerated. Indeed, the high NaCl concentrations enabled to increase the osmotic pressure difference between feed (liquid fraction from fermentation broth) and draw solutions thereby enabling a high water flux.

The FO experimental set up consisted of a counter-current configuration similar to previous studies [45]. The feed solution was faced against the active side of the membrane and the cross flow rate was adjusted with a variable-speed peristaltic pump (Longer BT100, 2 pump heads YZ1515X) at a constant flow rate of 200 mL min^{-1} . The draw solution reservoir was placed over a magnetic stirrer for constant mixing and a digital scale (Kern Balance 572, + software balance connection 4) recorded the feed solution weight change. The osmotic pressure difference between the feed and draw solutions was measured with an osmometer (Genotec Osmomat 030 Cryoscopic). All FO tests were performed at room temperature. The reverse salt flux was qualitatively assessed by measuring the conductivity change of the feed and draw solutions at the beginning and at the end of the FO test, respectively.

2.5 Analytical methods

The composition of the fermentation broths and FO samples (concentrated feed solution) were analyzed by HPLC (Ultimate 3000, Thermo Scientific) which contains a refractive index detection, equipped with a resin based sulfonated divinyl benzene-styrene-hydrogen column (Aminex HPX – 87H300 x 7.8 mm, BIO-RAD), under isocratic conditions and H_2SO_4 acid (5.0 mM) as eluent. The detection of the chemicals (sugars, VFA and ethanol) is achieved by

refractive index detection using a RefractoMax 521 (ThermoScientific) operated at 35 degrees.

The flow in the HPLC was maintained constant at 0.6 mL min⁻¹, the oven temperature at 63.5 °C.

2.6 Calculations

The Van't Hoff equation was used to calculate the osmotic pressure:

$$\Pi = iMRT \quad (1)$$

Where i is the Van't Hoff factor, M (mol L⁻¹) the molar concentration of the solution, R (atm L mol⁻¹ K⁻¹) is the ideal gas constant and T (K) the temperature.

The water flux, J_w (L m⁻² h⁻¹) was quantified by measuring the amount of water transported from the feed to the draw solution over time, and calculated by the equation:

$$J_w = \frac{\Delta V_f}{\rho A \Delta t} \quad (2)$$

Where, ΔV_f represents the change in weight (g) of feed solution; ρ is the density of water (g L⁻¹), A is the surface side (m²) of the membrane and Δt (h) accounts for the difference in time between measurements.

In order to assess the overall performance of FO technology, the indicators by Blandin et al. [46] were used, i.e. the solute concentration factor (SCF) and the water concentration factor (WCF).

$$SCF = \frac{C_{solute,f}}{C_{solute,0}} \quad (3)$$

$$WCF = \frac{V_{feed,0}}{V_{feed,f}} \quad (4)$$

$V_{feed,0}$ and $V_{feed,f}$ represent the initial and final volume (L) of the feed solution, $C_{solute,0}$ and $C_{solute,f}$ represent the concentration (g L^{-1}) of the solute at the beginning and at the end of the FO test, respectively.

The rejection rate of the membranes was calculated based on the expression by Engelhardt et al. [45]:

$$Rejection = 1 - \frac{V_{ds} C_{ds}}{V_w \frac{C_{f0} + C_{fe}}{2}} 100 \quad (5)$$

Where V_{ds} and C_{ds} represent the final volume (L) of the draw solution and the concentration of solute (g L^{-1}) in the draw solution, V_w is the volume (L) of water transported through the membrane, C_{f0} and C_{fe} represent the concentration (g L^{-1}) of solute in the feed solution at the beginning and at the end of the FO test.

3 Results and discussion

3.1 Fermentation trials

The fermentation of macroalgal hydrolysate reached a succinic acid titer of $36.81 \pm 0.32 \text{ g L}^{-1}$ and low titers of by-products (**Fig. 1a**), suggesting the potential of this substrate for succinic acid production [39]. On the other hand, the fermentation of the liquid stream from biopulp resulted in a lactic acid titer of $14.98 \pm 0.76 \text{ g L}^{-1}$ (**Fig. 1b**). Yet, the presence of glucose and xylose ($17.00 \pm 0.90 \text{ g L}^{-1}$) after 27 h of fermentation clearly indicates that the acidogenic potential was not fully exploited. The fermentation profile evidences that there was a slow degradation during the first 21 h, which resulted in a slight decrease of glucose and xylose concentrations. This could be attributed to the lack of acclimation of *Lactobacillus delbrueckii* to the culture conditions and the competition with the fermentative lactic acid bacteria (LAB) already present in the indigenous microcosmos. This might suggest that a longer fermentation time is needed in

order to increase the lactic acid titer. Additionally, 2.25 ± 0.16 and 3.27 ± 0.28 g L⁻¹ of succinic and acetic acid were produced, respectively.

Diluted biopulp fermentation enabled to obtain ethanol concentration titers of 13.94 ± 0.44 and 19.11 ± 0.51 g L⁻¹, respectively (**Fig. 1 c, d**). It should be highlighted that metabolic routes of *L. delbrueckii* enable to produce ethanol as main fermentation product from mixed sugar fermentation, which occurs through pyruvate and acetyl-coA production [47]. After fermentation trials, fermentation broths were centrifuged and the liquid fractions were used to perform the forward osmosis experiments.

3.2 Forward osmosis (FO) experiments

3.2.1 FO water flux and water removal

As shown in **Fig. 2 b**, 5.0 M NaCl draw solution promoted the highest water flux, from 12.52 L m⁻² h⁻¹ with the lactic acid broth to 22.06 L m⁻² h⁻¹ with the ethanol broth at the beginning of the tests. These values were higher than the water flux range obtained by Law et al. [16] with cellulose triacetate based flat sheet membranes. Due to the dilution effect in the feed solution and the net osmotic pressure loss, the water flux tended to decline with time [16] which resulted in a negligible flux after 30 min of operation. Due to the high specific area of the membranes, the FO tests could be concluded in a shorter time, when compared to time (> 12 h) required with flat sheet membranes [16]. The highest water flux exhibited by the ethanol broth might be related to the specific interaction of the chemicals present in the broth, with the membrane. A preliminary study with binary synthetic mixtures suggested that the water flux of thin film hollow fibre membranes was related to the molecular size of the chemicals and their speciation in ionic or free acid form (data not shown). Concretely, the ethanol broth contained the lower molecular weight

chemicals, i.e. HFor, HAc and EtOH, and low amounts of HLac and HSuc, which might be related to highest water flux.

FO test with lactic acid liquid broth exhibited the lowest water flux, which accounted for $4.26 \pm 3.4 \text{ L m}^{-2} \text{ h}^{-1}$, during the first 30 min using NaCl 1.5 M as draw solution (**Fig. 2a**). In this scenario, liquid fraction of the fermentation broth contained high amounts of lactic acid, glucose and xylose which are the highest molecular weight chemicals of the evaluated broths, and which evidenced their interaction with the membrane hindering the water flux. Size exclusion might have exerted a marked effect in the separation mechanism, where the lower molecular weight chemicals hindered the mass transfer and the water flux. This is in line with the results of Engelhardt et al. [45] who reported different water fluxes for different types of organic compounds, namely, the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), the plastic component bisphenol A (BPA) and the preservative methyl paraben. In addition, this broth contained high amounts of impurities, i.e. coloring organics, that were adsorbed in the membrane and it is suggested that contributed to lower the water flux [48]. The remaining sugars can be removed from the liquid fraction by adsorption materials, such as activated carbon as reported by [16]. The water removal obtained with NaCl 1.5 M, ranged within 35 – 72% (**Fig. 2c**) and as expected it was enhanced by increasing the osmotic pressure difference between the feed and draw solution [16]. As shown in **Table 3** increasing the draw solute concentration enabled to reach a higher osmotic pressure difference between the feed and draw solution -for example 155.5 bar with the succinic acid broth -which resulted in a high water removal from the fermentation broths. The final volumes of the ethanol and the succinic acid solutions were 97.10 and 149.78 mL respectively, where a water removal of 90.3% and 85% was achieved (**Fig. 2d**). The lowest water removal attained was with the lactic acid fermentation broth with 67.4% water

removal. Overall, the high water flux and high water removals linked to the higher osmotic pressure difference obtained when increasing the draw solute concentration suggested a promising scenario, which needs to be validated with the rejection rate of the membranes.

3.2.2 Rejection rate, up-concentration and reverse salt flux

A 100% rejection of the target chemicals was achieved for each of the forward osmosis tests. This was confirmed by means of the composition analysis of the concentrated draw solutions after completion of each FO experiments. For both draw solution compositions 1.5 and 5.0 M, succinic acid, lactic acid, and ethanol compositions were below the detection limit of the method.

Titers of ± 2.7 , 26.8 ± 1.1 , 34.6 ± 1.5 , and g L^{-1} for succinic acid, lactic acid, and ethanol, respectively were attained with NaCl 1.5 M draw solution (**Fig. 3a, c, e**). Nonetheless, these titers were significantly increased when the osmotic pressure difference between feed (fermentation broths) and draw solutions was raised. Herein, lactic acid could be concentrated up to $57.7 \pm 2.3 \text{ g L}^{-1}$ (**Fig. 3d**). A titer of $109.6 \pm 5.0 \text{ g L}^{-1}$ was achieved for ethanol (**Fig. 3e**) and succinic acid was up-concentrated up to $186.7 \pm 9.3 \text{ g L}^{-1}$ (**Fig. 3b**). Surprisingly, a high ethanol rejection was observed, which might be related to a compensation effect of additional anions had an influence in the rejection mechanisms, and contributed to retain ethanol in the feed side. At pH 7.1 all the anionic form of the chemicals is favored beyond their pK_a (**Table 4**), and thus electrostatic repulsions occur with the negative charged thin film hollow fibre membrane [49].

In addition, FO technology showed the potential to up-concentrate other secondary fermentation products. Acetic acid could be up-concentrated up to $41.1 \pm 2.0 \text{ g L}^{-1}$ in the succinic acid broth (**Fig. 3b**) and formic acid titer of $16.4 \pm 0.8 \text{ g L}^{-1}$ (**Fig. 3e**) was obtained during the ethanol broth

up-concentration with NaCl 5.0 M draw solution. These are additional valuable chemicals from the carboxylic platform with potential applications and market opportunities [50]. Besides, the unconverted sugars present in the fermentation broths were also up-concentrated, i.e. xylose concentration was increased up to $36.7 \pm 1.7 \text{ g L}^{-1}$ (**Fig. 3d**).

According to the conductivity measurements of the samples at the beginning and the end of FO tests, a reverse salt flux occurred in all tests with NaCl 1.5 M draw solution. Herein, an increased in conductivity of 1.26, 1.20, and 2.0 folds for succinic acid, lactic acid, and, ethanol fermentation broths respectively was recorded (**Fig. 4a**). In contrast, increasing the draw solute concentration did not translate into a higher reverse salt flux. Specifically, during lactic acid fermentation broth FO tests the reverse salt flux was negligible and only 1.0% increase in the salt content was recorded during succinic acid fermentation broth FO test. Contrarily, the ethanol fermentation broth FO test exhibited the highest reverse salt flux with a 57% increase in conductivity (49.3 mS cm^{-1}) in the up-concentrated broth (**Fig. 4b**).

The reverse salt flux competes with the direct water flux [33]. Thus, it might be expected that a higher water flux contributes to hinder the reverse salt flux. However, the results show a lower reverse salt flux in the lactic acid broth FO test. This might be related to the specific interaction of the ions present in the lactic acid broth, i.e. lactate, and the associated repulsion with Cl^- anions. In addition, chemical adsorption in the membrane, might help hinder the reverse salt flux [16]. In contrast, the ethanol broth contained the weakest and lower molecular weight ions, such as formate and acetate, which might exert a weaker repulsion effect than succinate and lactate ions.

3.2.3 Remarks on forward osmosis (FO) downstream

Forwards osmosis (FO) is an emerging technology that has been used for desalination and wastewater reclamation [51], but which has been barely explored and exploited as fermentation product downstream. Overall, the results of this study evidence the high potential of FO to remove water from fermentation broths. This can constitute a significant step in the subsequent downstream, contributing to obtain product titers that fall in the commercial range and enhancing the limitation of anaerobic fermentation processes. Of utmost importance for the economy of process is the energy and water consumption during the downstream processing as these are directly linked to the product titer in the fermentation broth. For instance, significant improvements in Cargill's lactic production facility have been achieved in terms of water consumption. Boost in lactic acid titer by 19.7% resulted in an overall abatement in water consumption of 21% [52].

Assessing all the parameters, the highest water concentration factor (i.e. highest water removal) and highest solute concentration factor are desirable. Overall, increasing the NaCl concentration, enabled to increase the solute concentration factor up to 4.0 – 5.5 along with a water concentration factor in the order of 3.60 – 6.75 were obtained. These values are in line with the values reported by Law et al. [16], where a succinic acid concentration factor of 3.9 was reported using a flat sheet cellulose triacetate membrane. However, during their study, the authors performed a pretreatment with activated carbon to remove residual compounds and clarify the fermentation broth. In this study, best results were obtained with the liquid fraction of the succinic acid fermentation broth obtained after centrifugation to remove biomass and cell debris, and straightforward FO technology processing with a thin film hollow fibre membrane. Concretely, a water concentration factor of 6.88 and a solute concentration factor of 5.33 were

obtained, which resulted in a succinic acid titer of $186.7 \pm 9.3 \text{ g L}^{-1}$. The high concentrated succinic acid mixture can be further purified by removing volatile fatty acids, i.e. formic acid and acetic acid, by vacuum distillation and by direct crystallization [16,53]. By decreasing the pH of the mixture to 3.0, and cooling down the mixture during 12 h at $5.0 \text{ }^{\circ}\text{C}$, a succinate salt precipitate was obtained (**Fig 5b**).

It is important to emphasize that high fermentation yields and product titers are desirable. For example, during lactic acid up concentration, the initial concentration $14.32 \pm 0.7 \text{ g L}^{-1}$ was remarkably enhanced, with a solute concentration factor of ca. 4.0 corresponding to a titer of $57.67 \pm 2.3 \text{ g L}^{-1}$. However, this concentration might not be enough for commercial purposes. Contrarily, and based on the results of FO tests, at least a solute concentration factor of 4.0 could be expected with FO membranes, where for example an initial product concentration of 40 g L^{-1} might lead to $\geq 160 \text{ g L}^{-1}$. Current fermentation yields and product titers greatly depend on the bacterial strains employed and the fermentation process conditions [52]. For example, Srivastava et al. [54] were able to obtain a lactic acid concentration of 84.5 g L^{-1} from cane molasses with *Lactobacillus delbrueckii* NCIM 2025 strain, during 12 h fermentation. Remarkably, engineered bacterial strains have enhanced succinic acid production yield from glucose, beyond 0.75 g/g yield and succinic acid concentration ranging within $40 - 107 \text{ g L}^{-1}$ [13]. Nonetheless, it is important to adapt current fermentation processes to real organic waste streams and to make current processes, technically feasible, cost-effective and scalable. For example, Lam et al. [55] reported a positive economic scenario for succinic acid production from bakery waste in a pilot scale facility.

Another important factor is the mass lost during the downstream process. Mass balance calculations indicate (data not shown) that significant amounts of ethanol were lost during FO

downstream, i.e. 17% when NaCl 5.0 M was used as draw solution. This was also observed in the preliminary tests performed with synthetic binary mixtures (data not shown), where 42.5% was lost. In contrast, the mass lost during the FO test of lactic acid fermentation broth was negligible and < 4% was lost during succinic acid FO test. Thus, additional alternatives for ethanol recovery should be considered to minimize the amount of solute evaporated.

In this study, best results were obtained with NaCl 5.0 M as draw solution, which resulted feasible at laboratory scale, but which might include higher draw solution consumption at pilot/demonstration scale, making the FO downstream unfeasible. Many draw solutions have been evaluated for FO application, which include different salts and organic compounds [56,57]. Herein, other process configurations might enable to reduce the osmotic pressure requirement, for example by continuous supply of the draw solution and minimizing the dilution effect. Interestingly, Blandin et al. [46] evaluated seawater and desalination brine for volatile fatty acid up grading, where high solute concentration factors were reported. It is noteworthy that the desalination brine constitutes a major management challenge at desalination facilities, associated to high discharge cost [58]. With a high saline content and with an estimated production of $142 \text{ M m}^3 \text{ d}^{-1}$, this waste stream constitutes a potential resource that could be tested as draw solution. Herein, further studies are required regarding the application of different draw solutes in FO downstream, where the influence of draw solute in the water flux, reverse salt flux and product(s) concentration need to be carefully assessed. Another promising candidates as draw solutes are the so-called deep eutectic solvents (DEPs). Due to their physical, chemical, thermal properties, low cost and biodegradability DESs represent a viable option for a variety of applications [59,60]. Pure DESs can generate high osmotic pressure ($\pi > 300 \text{ atm}$) and

significant osmotic pressure retention ($\pi > 60$ atm) even after 80% dilution, which make them promising candidates for maintaining high osmotic flux [61].

4 Conclusions

The results obtained in this study clearly confirmed the potential of thin film composite hollow fibre FO membrane to upgrade fermentation products of commercial relevance. FO was applied to the liquid fraction of the fermentation broths, which contained succinic acid, lactic acid and ethanol, respectively. The target chemicals could be significantly upgraded with solute concentration factors in the range of 4 - 5.5. High water removals were obtained using NaCl 5.0 M as draw solution. Promising results were obtained for the up-concentration of succinic acid from the fermentation broth, for which a product titer of 186.7 g L⁻¹ and water removal of 85% were obtained.

Credit Author Statment

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure captions

Fig. 1. Fermentation profiles for a) succinic acid production from macroalgal hydrolysate after 48 h with *Actinobacillus succinogenes* 130Z, b) lactic acid production from residual liquid biopulp after 27 h with *Lactobacillus delbrueckii*, c) and d) ethanol production from diluted biopulp after 14 h with *Lactobacillus delbrueckii*.

Fig. 2. Forward osmosis operation performance for the different fermentation broths, a, b) water flux and c, d) water removal.

Fig 3. Forward osmosis up-concentration results for fermentation broths.

Fig 4. Conductivity results for the different FO tests.

Fig 5 a) solute concentration factor (SCF) and water concentration factor (WCF) of the target solutes, respectively, and b) crystallization of succinate salt.

Tables

Table 1. Applications and market share of lactic acid, succinic acid and ethanol

Chemical	Applications	Market share	References
Lactic acid	Food additive (acidulant, preservative, emulsifier); biopolymer (polylactic acid - PLA); chemical intermediates (acrylic acid, propylene glycol, 2, 3 pentanedione, 1, 2 – propanediol acetaldehyde, pyruvic acid, lactide, etc.); esters (alkyl lactates)	Demand: 1960 kt by 2025 Market size; US\$ 8.77 billion	Alves de Oliveira et al. [10]; Dusselier et al. [63]; Varadarajan and Miller, [62]; Research and markets [64]
Succinic acid	Solvents (1-4 butanediol, tetrahydrofuran, 2-purrolidone, adipic acid, gamma-butyrolactone); linear aliphatic esters; biodegradable polymers (polybutylene succinate – PBS)	Market size: US\$ 131.73 million in 2018, projected to reach US\$ 182.84 million by 2023, at a CAGR of 6.8%	Jiang et al. [11]; Sreedevi et al. [65]
Ethanol	Transport fuel, power generation fuel, fuel for fuel cells, feedstock in the chemical industry	Market size: US\$ 77.74 billion in 2018, projected to reach US\$ 122.35 billion by 2026, at a CAGR of 5.6%	Reports and data [66]

Table 2. Composition of the substrates and fermentation broths

	Macroalgal hydrolysate*	Succinic acid broth*	Biopulp liquid fraction**	Diluted Biopulp (1:1)
Glucose (g L ⁻¹)	28.23 ± 1.02	0.63 ± 0.02	15.07 ± 0.51	1.52 ± 0.15
Xylose (g L ⁻¹)	0.00 ± 0.00	0.00 ± 0.00	22.48 ± 0.89	0.00 ± 0.00
Mannitol (g L ⁻¹)	12.00 ± 0.37	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Arabinose (g L ⁻¹)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
HSuc (g L ⁻¹)	0.43 ± 0.01	36.81 ± 0.32	0.00 ± 0.00	0.23 ± 0.01
HLac (g L ⁻¹)	1.99 ± 0.08	4.20 ± 0.13	12.30 ± 0.17	8.09 ± 0.44
HFor (g L ⁻¹)	0.00 ± 0.00	1.36 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
HAc (g L ⁻¹)	0.00 ± 0.00	7.93 ± 0.06	0.00 ± 0.00	1.52 ± 0.00
EtOH (g L ⁻¹)	0.00 ± 0.00	0.96 ± 0.23	0.00 ± 0.00	3.51 ± 0.01

*Obtained from Marinho et al. [9]; **Liquid fraction from municipal biopulp after being separated by centrifugation

Table 3. Experimental set up for the forward osmosis experiments

Feed solution	NaCl concentration (M)	ΔP initial (bar)	pH of feed solution	ΔP final (bar)	Time (min)
Succinic acid broth	1.5	43.2	7.50	0.0	45
	5.0	155.5		75.06	30
Lactic acid broth	1.5	45.3	5.90	0.0	45
	5.0	148.2		11.50	30
Ethanol broth ²	1.5	N.A. ¹	7.10	N.A. ¹	45
	5.0	N.A. ¹		N.A. ¹	30

¹Crystallization errors of the sample prevented any possible measurement in the osmometer.

²The ethanol broth was the mixture of both fermentation outcomes obtained during diluted biopulp fermentation.

Table 4. Chemical properties of the different compounds present in the fermentation broths

Compound	HFor	EtOH	HAc	HLac	HSuc	Xylose	Glucose
Formula	CH ₂ O ₂	C ₂ H ₅ OH	C ₂ H ₄ O ₂	C ₃ H ₆ O ₃	C ₄ H ₆ O ₄	C ₅ H ₁₀ O ₅	C ₆ H ₁₂ O ₆
Molecular weight (g mol ⁻¹)	46.02	46.07	60.05	90.08	118.09	150.13	180.16
<i>pK_a</i>	4.60	15.9	4.76	3.86	4.21, 5.63	12.14	N.A.